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Antioxidant and antimicrobial activity evaluation of polyhydroxycinnamic acid ester derivatives†

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Polyhydroxycinnamic acid esters **5a-p** have been synthesized starting from the appropriately substituted benzaldehydes. The antioxidant activity of these esters has been determined by superoxide free radical scavenging activity and DPPH free radical scavenging activity. The SAR studies reveal that pyrogallol, catechol moieties are essential for good antioxidant activity and an increase in the length of alkyl chain of the ester decreases the activity. Butyl hydroxycinnamates exhibit higher antibacterial activity among the synthesized hydroxycinnamates **5a-p**, but, none of these show significant antifungal activity.

Keywords: Polyhydroxycinnamates, synthesis, antioxidant, antimicrobial, SAR studies

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Hydroxycinnamic acid esters are widely distributed in plant kingdom and are reported as antioxidants[1-3]. These compounds impart nutriceutical traits to foods by way of their abilities to serve as cellular anti-oxidants, anti-inflammatory agents or inhibitors of enzymes involved in cell proliferation. These activities are important in ameliorating chronic diseases such as cancer, arthritis, and cardiovascular diseases, which in some cases may be caused by free radicals[4].

Free radicals have also been implicated in a number of pathological processes, which include aging, inflammation, reoxygenation of ischemic tissues, atherosclerosis, and various kinds of cancer. The harmful free radicals such as hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$), and the superoxide anion (O_2^{-}) are constantly being produced as a result of metabolic reactions in living systems. Living systems are protected from oxidative damage of these reactive species by enzymes such as superoxide dismutase and glutathione peroxidase. Antioxidant compounds such as ascorbic acid, tocopherols, and carotenoids[4] and natural phenolic compounds have been reported to remove free radicals and protect the structural integrity of cells and tissues[5-8].

Several synthetic methods are available for the synthesis of cinnamic acid esters and the important methods are: (i) Esterification of corresponding cinnamic acids with alcohols using the reagents like DCC, acids[9,10], (ii) Wittig reaction[11,12] between aldehydes and triphenylphosphoranes and the modified procedure, Wittig-Horner sonochemical reaction[13] conditions, and (iii) Reformatsky reaction[14] of α-haloesters with aromatic aldehydes or ketones in presence of zinc and diethylaluminum chloride.

In view of the importance of natural antioxidants, we have carried out synthesis and the evaluation of antioxidant activity of a series of cinnamic acid esters **5a-p** having hydroxyls, methoxyls on the benzene ring and methyl (C_1, **5a**, **5e**, **5g**, **5k** and **5m**), 1-butyl (C_4, **5b**, **5f**, **5h**, **5l** and **5n**), 1-tetradecyl (C_{14}, **5c**, **5i** and **5o**), 1-eicosanyl (C_{20}, **5d**, **5j** and **5p**) as alkyl part to obtain structure-activity relationships. The anti-oxidant activity of these compounds was determined by superoxide free radical scavenging activity using nitroblue tetrazolium (NBT) method and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and the results are presented in this paper.

Further, the antimocrobial activity was also screened for these compounds against the Gram-negative organisms (*Escherichia coli, Pseudomonas*

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aeruginosa), Gram-positive organisms (*Bacillus subtilis, Staphylococcus aureus*), *Aspergillus wentii* and *Aspergillus niger*.

Results and Discussion

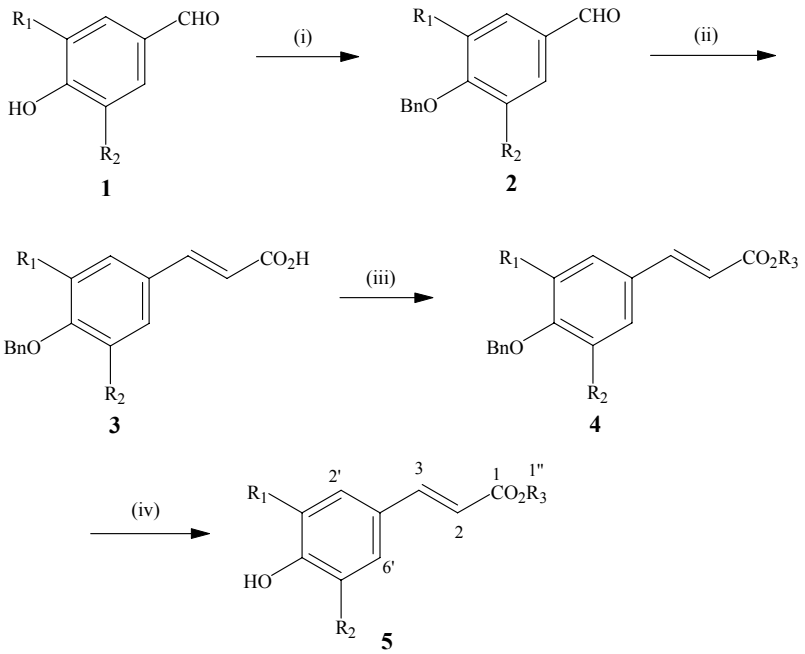
Synthesis

The desired cinnamic acid esters have been synthesized by the reaction of appropriately substituted benzaldehydes with malonic acid followed by esterification.

The hydroxyl groups on the starting benzaldehydes **1** were protected as benzyl ethers using benzyl bromide and K_2CO_3 as a base[15]. Condensation of benzyloxybenzaldehydes **2** with malonic acid under Knoevengal-Doebner conditions[16], gave the corresponding benzyloxycinnamic acids **3**. The acids **3** were converted into the corresponding esters **4** by the standard esterification methods[17] using appropriate alcohols. Debenzylation of these esters **4** using $AlCl_3$ and N,N-dimethylaniline[18] (**Scheme I**) gave the desired polyhydroxycinnamic acid esters **5a-p**. The structures of these esters **5a-p** were confirmed by their physical and spectral (IR, NMR and mass) data.

Antioxidative activity

The antioxidant activity of the esters **5a-p** was determined by two different mechanisms: (a) Superoxide free radical scavenging ability (NBT method)[19,20], and (b) DPPH free radical scavenging activity[21]. The IC_{50} values of the esters are noted in **Table I**. From the superoxide radical scavenging data, it is evident that an increase in the number of hydroxyls on the aromatic ring enhanced the antioxidant activity (**5a**, IC_{50} 465 μM; **5g**, IC_{50} 19 μM; **5m**, IC_{50} 4 μM), whereas the increase in the ester chain length diminishes the antioxidant activity (**5m**, IC_{50} 4 μM; **5n**, IC_{50} 4 μM; **5o**, IC_{50} 91 μM; **5p**, IC_{50} 108 μM). Other oxygenated substitutions like OCH_3



1 **2** **3** **4** **5**

Compd	R_1	R_2	R_3	Compd	R_1	R_2	R_3
5a	H	H	CH_3	**5i**	OH	H	$C_{14}H_{29}$
5b	H	H	C_4H_9	**5j**	OH	H	$C_{20}H_{41}$
5c	H	H	$C_{14}H_{29}$	**5k**	OCH_3	OCH_3	CH_3
5d	H	H	$C_{20}H_{41}$	**5l**	OCH_3	OCH_3	C_4H_9
5e	OCH_3	H	CH_3	**5m**	OH	OH	CH_3
5f	OCH_3	H	C_4H_9	**5n**	OH	OH	C_4H_9
5g	OH	H	CH_3	**5o**	OH	OH	$C_{14}H_{29}$
5h	OH	H	C_4H_9	**5p**	OH	OH	$C_{20}H_{41}$

(i) Benzyl bromide, K_2CO_3, acetone, reflux, 4 hr, 85-90% (ii) $CH_2(COOH)_2$, pyridine, piperidine, 80-90°C, 3 hr, 90-95% (iii) H^+, R-OH, reflux, 2 hr, 60-80% (iv) $AlCl_3$, N,N-dimethylaniline, 50-60%.

Scheme I

Table I — Antioxidant activity of polyhydroxycinnamic acid esters **5a-p**

Compd	Superoxide scavenging activity (IC_{50} μM)	DPPH radical scavenging activity (IC_{50} μM)
5a	465	>100
5b	447	>100
5c	416	>100
5d	477	>100
5e	399	41
5f	512	51
5g	19	10
5h	32	11
5i	68	11
5j	98	13
5k	205	31
5l	571	38
5m	4	9
5n	4	9
5o	91	9
5p	108	10
BHA	966	18
BHT	381	19
Vitamin C	852	14
Vitamin E	726	>500

also contribute positively to the antioxidant activity, as illustrated by increase in antioxidant activity with the increase in the number of OCH_3 groups. But, the contribution from OCH_3 is markedly low compared to that from a free phenolic group (**5e**, IC_{50} 399 μM and **5g**, IC_{50} 19 μM). Other subgroups also show similar trend in activity variation with chain length of alkyl part (**5a-d**, **5e**, **5f**, **5g-j**, **5k** and **5l**). Interestingly, the trihydroxycinnamates **5m-p** synthesized for the first time showed higher antioxidant activity compared to the commercially available antioxidants like BHA, BHT, vitamin-E and vitamin C (**Table I**).

Antibacterial activity

The antibacterial activity of the hydroxycinnamates **5a-p** was carried out by the cylinder-plate (agar-cup plate) diffusion method[22] against the Gram-negative organisms (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive organisms (*Bacillus subtilis, Staphylococcus aureus*).

The data (**Table II**) revealed that *n*-butyl esters of the cinnamic acids exhibited potent antimicrobial activity among the hydroxycinnamates. Dihydroxy and trihydroxy butyl cinnamates (**5h** and **5n)** exhibited strongest antimicrobial activity (15.5 mm) against the Gram-negative organism *B. subtilis* at a concentration of 500 μg/0.05 mL. The long chain, *n*-tetradecyl and *n*-eicosanyl, esters of the hydroxycinnamates did not exhibit any significant activity even at a concentration of 500 μg/0.05 mL.

Table II — Antibacterial activity of polyhydroxycinnamates **5a-p** at a concentration of 500 μg/ 0.05 mL
Zone of inhibition (mm)

Compd	*E. coli*	*P. aeroginosa*	*B. subtilis*	*S. aureus*
5a	11.5	—	10.5	8.0
5b	12.0	12.5	13.5	14.0
5f	10.5	9.5	12.5	10.0
5g	12.5	—	13.0	14.0
5h	14.0	—	15.5	—
5l	8.0	8.0	8.0	10.5
5m	11.0	13.0	10.5	12.0
5n	13.0	13.0	15.5	14.5

— No significant antibacterial activity

Antifungal activity

The hydroxycinnamates **5a-p** were screened for their antifungal activity against the organisms, such as *Aspergillus wentii* and *Aspergillus niger*, by cylinder-plate method (agar cup-plate)[22], as described for the evaluation of antibacterial activity. The study revealed that hydroxycinnamates do not possess any significant antifungal activity even at a concentration of 500 μg/ 0.05 mL.

Materials and Methods

The test organisms for the antimicrobial activity studies, *Escherichia coli, Pseudomonas aeruginosa* (Gram-negative), *Bacillus subtilis, Staphylococcus aureus* (Gram-positive), *Aspergillus wentii* and *Aspergillus niger* were obtained from National Collection of Industrial Microorganism, Pune, India.

Experimental Section

Melting points were recorded on a V Scientific melting point apparatus, in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer BX1 FTIR spectrophotometer; ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra on a Varian Gemini 400 MHz NMR spectrometer (chemical shifts in δ, ppm and coupling constants, J in Hz); and mass

spectra on Agilent 1100 Series LC/MSD. The elemental analysis was carried out on a Vario El Elementar instrument. Column chromatography was carried out using ACME silica gel (100-200 mesh/finer than 200 mesh).

General procedure for the preparation of benzyloxybenzaldehydes 2. A mixture of hydroxybenzaldehydes (10 mmoles), benzyl bromide (13 mole equivalents for each OH group), potassium carbonate (20 mole equivalents for each OH group) and acetone (25 mL) was heated under reflux for 4 hr. After completion of reaction, the solid was filtered off and the solvent was evaporated *in vacuo*. The residue was diluted with cold water and extracted with diethyl ether. The combined ether layer was washed with water, brine and dried over sodium sulfate. The residue obtained after removal of the solvent was purified by silica gel column chromatography to give the benzyloxybenzaldehydes **2** (yield 85-90%).

General procedure for the preparation of benzyloxycinnamic acids 3. A mixture of benzyloxybenzaldehyde **2** (5 mmoles), malonic acid (12 mmoles), pyridine (30 mmoles) and piperidine (0.25 mL) was heated on a water-bath (90-95°C) for 3 hr. The reaction mixture was heated under reflux for further 5 min and allowed to cool to rt. The cooled reaction mixture was poured into excess dil. HCl (50 mL, 2*N*). The precipitated solid was filtered, washed with cold water and dried to give the corresponding cinnamic acids **3** (yield 90-95%).

General procedure for the preparation of benzyloxycinnamic acid esters 4. A mixture of the acid **3** (5 mmoles), alcohol (20 mL), and sulfuric acid (1 mL) was refluxed for 2 hr. The cooled reaction mixture was poured into cold water and extracted with chloroform. The chloroform layer was washed with water, 10% sodium bicarbonate, brine and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column to give the corresponding esters **4** (yield 60-80%).

General procedure for the debenzylation of esters 4 into 5. To a mixture of the benzyloxycinnamate **4** (1 mmole), N,N-dimethylaniline (3 mole equivalents for each benzyloxy group) and dichloromethane (25 mL) was added aluminum chloride (2 mole equivalents for each benzyloxy group) at 0°C and the reaction mixture was stirred at 0-5°C for 2 hr. The reaction mixture was quenched with 1*N* HCl (30 mL) and extracted with ethyl acetate. The organic layer was washed with 10% sodium bicarbonate, brine and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column to give the corresponding esters **5** (yield 50-60%) and the physical and spectral data are presented below.

Methyl 4-hydroxycinnamate 5a: m.p. 138-40°C (lit.[23] m.p. 136°C); IR (neat): 3382, 1693, 1634, 1604, 986 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.92 (3H, s, H-1″), 5.73 (1H, s, Ar-OH), 6.43 (1H, d, *J*=16.0 Hz, H-2), 7.01 (2H, d, *J*=8.4 Hz, H-3′, 5′), 7.55 (2H, d, *J*=8.4 Hz, H-2′, 6′), 7.76 (1H, d, *J*=16.0 Hz, H-3); EIMS (%): m/z 179 ($M+H$, 9), 178 (M^+, 83), 147 (100), 120 (51), 91 (29) and 66 (22).

1-Butyl 4-hydroxycinnamate[24] 5b: m.p. 72-74°C; IR (neat): 3373, 2959, 1673, 1636, 1603, 978 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, *J*=7.4 Hz, H-4″), 1.32-1.41 (2H, m, H-3″), 1.58-1.65 (2H, m, H-2″), 4.14 (2H, t, *J*=6.7 Hz, H-1″), 5.96 (1H, s, Ar-OH), 6.23 (1H, d, *J*=16.0 Hz, H-2), 6.79 (2H, d, *J*=8.5 Hz, H-3′, 5′), 7.35 (2H, d, *J*=8.5 Hz, H-2′, 6′), 7.56 (1H, d, *J*=16.0 Hz, H-3); LCMS (ESI – Negative mode): m/z 219 $(M-H)^-$.

1-Tetradecyl 4-hydroxycinnamate 5c: m.p. 88-90°C; IR (neat): 3382, 2924, 1674, 1603 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, *J*=6.8 Hz, H-14″), 1.26-1.42 (22H, m, H-3″-13″), 1.64-1.73 (2H, m, H-2″), 4.19 (2H, t, *J*=6.7 Hz, H-1″), 5.56 (1H, s, Ar-OH), 6.30 (1H, d, *J*=15.9 Hz, H-2), 6.84 (2H, d, *J*=8.5 Hz, H-3′, 5′), 7.42 (2H, d, *J*=8.5 Hz, H-2′, 6′), 7.62 (1H, d, *J*=15.9 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 168.1, 158.1, 144.7, 130.0, 127.1, 116.0, 115.5, 65.0, 32.0, 29.7-28.8, 26.0, 22.7, 14.1; LCMS (ESI – Negative mode): m/z 359 $(M-H)^-$; Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.67; H, 10.00. Found: C, 76.65; H, 10.28%.

1-Eicosanyl 4-hydroxycinnamate[25] 5d: m.p. 90-92°C; IR (KBr): 3383, 2922, 1674, 1603, 981 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00 (3H, t, *J*=6.8 Hz, H-20″), 1.35-1.45 (34H, m, H-3″-19″), 1.78-1.86 (2H, m, H-2″), 4.31 (2H, t, *J*=6.7 Hz, H-1″), 5.44 (1H, s, Ar-OH), 6.43 (1H, d, *J*=16.0 Hz, H-2), 6.96 (2H, d, *J*=8.5 Hz, H-3′, 5′), 7.56 (2H, d, *J*=8.5 Hz, H-2′, 6′), 7.75 (1H, d, *J*=16.0 Hz, H-3); EIMS (%): m/z 444 (M^+, 17), 166 (45), 164 (100), 147 (52), 121 (24) and 107 (20).

Methyl 4-hydroxy-3-methoxycinnamate 5e: m.p. 58-60°C (lit.[26] m.p. 63-64°C); IR (KBr): 3398, 2946, 1701, 1638, 1602, 1680, 981 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.79 (3H, s, H-1″), 3.92 (3H, s, Ar-OCH_3), 5.91 (1H, s, Ar-OH), 6.29 (1H, d, *J*=15.9 Hz, H-2), 6.92 (1H, d, *J*=8.2 Hz, H-5′), 7.02 (1H, d, *J*=1.8 Hz, H-2′), 7.07 (1H, dd, *J*=8.2, 1.8 Hz, H-6′),

7.62 (1H, d, *J*=15.9 Hz, H-3); LCMS (ESI – Negative mode): m/z 207 $(M-H)^-$.

1-Butyl 4-hydroxy-3-methoxycinnamate[24] **5f**: m.p. 46-48°C; IR (neat): 3396, 2960, 1702, 1634, 1603, 980 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.96 (3H, t, *J*=7.4 Hz, H-4″), 1.39-1.48 (2H, m, H-3″), 1.64-1.72 (2H, m, H-2″), 3.92 (3H, s, Ar-OCH_3), 4.20 (2H, t, *J*=6.7 Hz, H-1″), 5.90 (1H, s, Ar-OH), 6.29 (1H, d, *J*=15.9 Hz, H-2), 6.91 (1H, d, *J*=8.2 Hz, H-5′), 7.03 (1H, d, *J*=1.7 Hz, H-2′), 7.07 (1H, dd, *J*=8.2, 1.7 Hz, H-6′), 7.60 (1H, d, *J*=15.9 Hz. H-3); LCMS (ESI – Negative mode): m/z 249 $(M-H)^-$.

Methyl 3,4-dihydroxycinnamate 5g: m.p. 156-58°C (lit.[27] m.p. 161-63°C); IR (neat): 3300, 1680, 1630, 1600, 960 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.92 (3H, s, H-1″), 5.69 (1H, s, Ar-OH), 5.79 (1H, s, Ar-OH), 6.39 (1H, d, *J*=16.0 Hz, H-2), 7.00 (1H, d, *J*=8.2 Hz, H-5′), 7.14 (1H, d, *J*=8.2 Hz, H-6′), 7.20 (1H, s, H-2′), 7.71 (1H, d, *J*=16.0 Hz, H-3); EIMS (%): m/z 194 (M^+, 73), 164 (44), 135 (54), 134 (100), 133 (94), 124 (5), 120 (4) and 110 (12).

1-Butyl 3,4-dihydroxycinnamate 5h: m.p. 112-14°C (lit.[27] m.p. 110-11°C); IR (neat): 3489, 2953, 1686, 1639, 1604, 974 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.96 (3H, t, *J*=7.4 Hz, H-4″), 1.38-1.48 (2H, m, H-3″), 1.65-1.72 (2H, m, H-2″), 4.21 (2H, t, *J*=6.7 Hz, H-1″), 6.16 (1H, br s, Ar-OH), 6.27 (1H, d, *J*=15.9 Hz, H-2), 6.45 (1H, br s, Ar-OH), 6.88 (1H, d, *J*=8.2 Hz, H-5′), 7.00 (1H, dd, *J*=8.2, 1.8 Hz, H-6′), 7.12 (1H, d, *J*=1.8 Hz, H-2′), 7.59 (1H, d, *J*=15.9 Hz, H-3); LCMS (ESI – Negative mode): m/z 235 $(M-H)^-$.

1-Tetradecyl 3,4-dihydroxycinnamate[28] **5i**: m.p. 116-18°C; IR (KBr): 3481, 2920, 1684, 1605, 975, 863 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.86 (3H, t, *J*=6.8 Hz, H-14″), 1.10-1.40 (22H, m, H-3″-13″), 1.59-1.64 (2H, m, H-2″), 4.11 (2H, t, *J*=6.6 Hz, H-1″), 6.25 (1H, d, *J*=15.9 Hz, H-2), 6.76 (1H, d, *J*=8.1 Hz, H-5′), 6.99 (1H, dd, *J*=8.1, 1.9 Hz, H-6′), 7.04 (1H, d, *J*=1.9 Hz, H-2′), 7.46 (1H, d, *J*=15.9 Hz, H-3), 9.10 (1H, br s, Ar-OH), 9.54 (1H, br s, Ar-OH); ^{13}C NMR (DMSO-d_6): δ 166.5, 148.4, 145.6, 144.9, 125.6, 121.2, 115.7, 114.8, 114.9, 63.7, 31.3, 29.1-28.2, 25.4, 22.1, 13.9; LCMS (ESI – Negative mode): m/z 375 $(M-H)^-$; Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.40; H, 9.57. Found: C, 73.87; H, 9.43%.

1-Eicosanyl 3,4-dihydroxycinnamate 5j: m.p. 112-14°C (lit.[15] m.p. 109-10°C); IR (KBr): 3479, 2919, 1683, 1605, 975 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, *J*=6.8 Hz, H-20″), 1.26-1.39 (34H, m, H-3″-19″), 1.68-1.71 (2H, m, H-2″), 4.18 (2H, t, *J*=6.7 Hz, H-1″), 5.49 (1H, s, Ar-OH), 5.63 (1H, s, Ar-OH), 6.27 (1H, d, *J*=15.9 Hz, H-2), 6.87 (1H, d, *J*=8.2 Hz, H-5′), 7.02 (1H, d, *J*=8.2 Hz, H-6′), 7.08 (1H, br s, H-2′), 7.56 (1H, d, *J*=15.9 Hz, H-3); EIMS (%): m/z 461 (M+H, 15), 460 (M^+, 47), 181 (54), 180 (28), 179 (100), 163 (56), 135 (18), 134 (10) and 123 (17).

Methyl 4-hydroxy-3,5-dimethoxycinnamate 5k: m.p. 92-94°C (lit.[29] m.p. 90-92°C); IR (neat): 3417, 1704, 1604, 1633, 830 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.79 (3H, s, H-1″), 3.91 (6H, s, 2 × Ar-OCH_3), 5.85 (1H, br s, Ar-OH), 6.29 (1H, d, *J*=15.9 Hz, H-2), 6.77 (2H, s, H-2′, 6′), 7.60 (1H, d, *J*=15.9 Hz, H-3); LCMS (ESI – Negative mode): m/z 237 $(M-H)^-$.

1-Butyl 4-hydroxy-3,5-dimethoxycinnamate[30] **5l**:: m.p. 84-86°C; IR (neat): 3419, 2960, 1703, 1634, 1603, 980 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.97 (3H, t, *J*=7.0 Hz, H-4″), 1.21-1.83 (4H, m, H-2″, 3″), 3.91 (6H, s, 2 × Ar-OCH_3), 4.21 (2H, t, *J*=7.0 Hz, H-1″), 6.30 (1H, d, *J*=15.9 Hz, H-2), 6.77 (2H, s, H-2′, 6′), 7.59 (1H, d, *J*=15.9 Hz, H-3); LCMS (ESI – Negative mode): m/z 279 $(M-H)^-$.

Methyl 3,4,5-trihydroxycinnamate[31] **5m**: m.p. 188-90°C; IR (KBr): 3392, 1673, 1605, 974 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.72 (3H, s, H-1″), 6.25 (1H, d, *J*=15.6 Hz, H-2), 6.74 (2H, s, H-2′, 6′), 7.46 (1H, d, *J*=15.6 Hz, H-3); ^{13}C NMR (acetone-d_6): δ 167.9, 146.7, 146.2, 136.7, 126.3, 115.2, 108.3, 51.5; LCMS (ESI – Negative mode): m/z 209 $(M-H)^-$.

1-Butyl 3,4,5-trihydroxycinnamate 5n: m.p. 146-48°C; IR (KBr): 3468, 2960, 1694, 1611, 969 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.92 (3H, t, *J*=7.4 Hz, H-4″), 1.33-1.42 (2H, m, H-3″),1.58-1.65 (2H, m, H-2″),4.12 (2H, t, *J*=6.6 Hz, H-1″), 6.16 (1H, d, *J*=15.8 Hz, H-2),6.60 (2H, s, H-2′, 6′),7.38 (1H, d, *J*=15.8 Hz, H-3); ^{13}C NMR (DMSO-d_6): δ 167.5, 145.6, 145.3, 136.0, 124.7, 114.4, 107.6, 64.0, 30.0, 18.5, 13.2; LCMS (ESI – Negative mode): m/z 251 $(M-H)^-$; Anal. Calcd for $C_{13}H_{16}O_5$: C, 61.90; H, 6.35. Found: C, 61.67; H, 6.28%.

1-Tetradecyl 3,4,5-trihydroxycinnamate 5o: m.p. 96-98°C; IR (KBr): 3476, 2922, 1668, 1609, 978, 835 cm^{-1}; ^1H NMR (acetone-d_6): δ 1.01 (3H, t, *J*=6.7 Hz, H-14″), 1.30-1.50 (22H, m, H-3″-13″), 1.78-1.85 (2H, m, H-2″), 4.27 (2H, t, *J*=6.7 Hz, H-1″), 6.35 (1H, d, *J*=15.8 Hz, H-2), 6.85 (2H, s, H-2′, 6′), 7.59 (1H, d, *J*=15.8 Hz, H-3); ^{13}C NMR (acetone-d_6): δ 167.4, 146.7, 145.8, 136.6, 126.7, 116.0, 108.5, 64.6, 32.6, 30.3-29.2, 26.6, 23.2, 14.3; LCMS (ESI – Negative mode): m/z 391 $(M-H)^-$; Anal. Calcd for $C_{23}H_{36}O_5$: C, 70.41; H, 9.18. Found: C, 69.98; H, 9.56%.

1-Eicosanyl 3,4,5-trihydroxycinnamate 5p: m.p. 110-12°C; IR (KBr): 3452, 2918, 1679, 1611, 976, 834 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.86 (3H, t, *J*=6.7 Hz, H-20″), 1.15-1.45 (34H, m, H-3″-19″), 1.60-1.64 (2H, m, H-2″), 4.10 (2H, t, *J*=6.7 Hz, H-1″), 6.15 (1H, d, *J*=15.8 Hz, H-2), 6.59 (2H, s, H-2′, 6′), 7.37 (1H, d, *J*=15.8 Hz, H-3); ^{13}C NMR (DMSO-d_6): δ 166.4, 146.1, 145.3, 136.4, 124.4, 114.0, 107.6, 63.6, 31.3, 29.5-28.7, 28.3, 25.4, 22.1, 13.8; LCMS (ESI – Negative mode): m/z 475 $(M-H)^-$; Anal. Calcd for $C_{29}H_{48}O_5$: C, 73.11; H, 10.08. Found: C, 72.50; H, 10.35%.

Biological Tests

Superoxide and DPPH radical scavenging activities of hydroxycinnamates **5a-p** were determined by the methods of McCord[19] and Wang *et al.*[21] respectively. The antimicrobial assay of the hydroxycinnamates was determined using the standard procedure[22].

Conclusions

The polyhydroxycinnamic acid esters **5a-p** were synthesized starting from the corresponding benzyl-oxybenzaldehydes in three steps. The antioxidant activity of these esters was determined by superoxide scavenging activity and DPPH free radical scavenging activity. The SAR studies reveal that pyrogallol and catechol moieties are essential for good antioxidant activity and an increase in the length of alkyl chain of the ester decreases the activity. The antimicrobial screening results suggest that the butyl hydroxy-cinnamates exhibited better antibacterial activity against the Gram-negative organism *B. subtilis* and no hydroxycinnamate exhibited significant antifungal activity against the microorganisms, *Aspergillus wentii* and *Aspergillus niger*, even at a concentration of 500 μg/0.05 mL.

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